PAPER ELECTROPHORESIS THROUGH DIALYSING BARRIERS

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INTRODUCTION

Paper eletrophoresis has been successfully used for the separation of low-molecular weight organic compounds, suchs as vitamins, sugars, polyalcohols, purine and pyrimidine bases, amino acids, etc., and of various inorganic compounds[1].

In many instances, however, such as when these compounds are being studied in blood specimens, the samples must be previously deproteinized. The usual deproteinization procedures must frequently be avoided, since they can lead to the loss of the compounds mentioned. Hence the technique of centrifugal–ultrafiltration was devised to separate unstable, low-molecular weight substances from blood proteins[2].

A further complication arises when the specimens to be examined are very small such as blood samples drawn from mice. In these cases deproteinization and electrophoretic separation of low-molecular weight compounds were achieved simultaneously by means of the intercalation of dialysing barriers in the electrophoresis paper strip.

MATERIALS AND METHODS

Paper electrophoresis was carried out by the hanging strip method, using a Shandon equipment. The conditions (buffer, voltage and time) were chosen according to the case.

The dialysing barriers are prepared as follows: a starting line is drawn with a soft pencil at the middle of a 4 × 37 cm filter paper (Macherey-Nagel No. 261) strip. Two other lines are drawn parallel to the starting line, at a distance of 0.5 cm from each side of it and similarly spaced from the borders of the strip. Along them a 1 mm slit is cut by means of a razor blade. Two drops of collodion (4% w/v in ethyl ether–ethanol, 3:1) are then left to run over the slits and the solvent is evaporated at room temperature for a few minutes; the apertures are thus covered with a semi-permeable membrane.

The paper strip so prepared is soaked in the chosen buffer for about half an hour, excess buffer is blotted out with filter paper and the sample to be studied is applied over the starting line, as usual. Four V-shaped segments are then cut from the paper

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at the end of the slits, leaving two 1 mm wide collodion bridges as the sole connections between the three segments of the strip, as can be seen in Fig. 1.

This set-up is placed in the electrophoretic chamber, and a chosen potential is

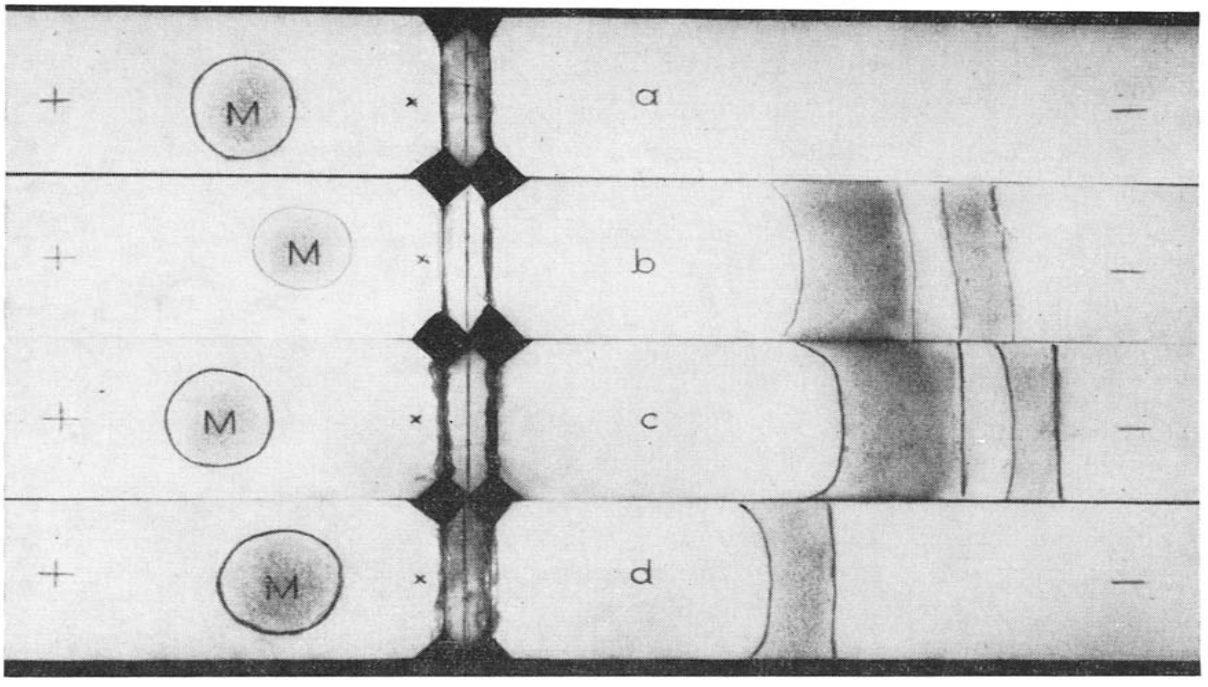

Fig. 1. Paper electrophoresis (veronal–acetate buffer, pH 9.0, 330 V/4 h) through dialysing barriers of (a) mouse serum, (b) streptomycin, (c) mouse serum plus streptomycin and (d) serum from a mouse previously injected with streptomycin. M = marker (bromothymol blue).

applied during a suitable time, which is usually double that needed for the same separation by routine methods. The collodion bridges act as dialysing barriers, through which only the electrically charged, low-molecular weight compounds will pass.

RESULTS AND DISCUSSION

The above method was tried successfully for the separation of sugars, amino acids and antibiotics from blood samples. Fig. 1 shows the result of the barrier-electrophoresis (veronal–acetate buffer pH 9: $u = 0.06/330$ Volts; 4 h) of mouse blood serum drawn 5 min after the intravenous injection of 4 mg streptomycin sulphate and prepared as described previously[3], comparing it with those found for a standard solution of streptomycin, a sample of serum from an untreated mouse and an identical sample with streptomycin added. Guanidine compounds were localized by the Sakaguchi reaction, as described by WU[4], and proteins were stained by bromophenol blue–zinc sulphate[5].

As the electrical conductivity of the dialysing barrier is not constant, a suitable marker (M) must be spotted on the paper strip, in order to evaluate the displacement of the compound being studied. In the present case a drop of an alcoholic solution of bromothymol blue (0.1% w/v) was spotted in the middle of the paper strip, 0.5 cm to the anode side of the barrier. Streptomycin and the dye migrate in opposite directions, and thus the antibiotic can be assayed colorimetrically free from interfering substances, after elution from the paper.

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SUMMARY

The technique of paper electrophoresis through dialysing barriers was devised to separate low-molecular weight, electrically charged compounds from proteins. It is based on the intercalation of collodion bridges in the electrophoresis paper strip, one at each side of the starting line, the displacement of proteins being thus barred while smaller molecules move freely.

REFERENCES

1 L. P. RIBEIRO, E. MITIDIERI AND O. R. AFFONSO, *Paper Electrophoresis*, Elsevier, Amsterdam, 1961.
2 R. C. R. BARRETO AND D. B. MANO, *J. Chromatog.*, 7 (1962) 82.
3 R. C. R. BARRETO, *J. Chromatog.*, 6 (1961) 278.
4 CHUNG WU, *Arch. Biochem. Biophys.*, 85 (1959) 461.
5 R. C. R. BARRETO AND L. A. D. TAVARES, *Rev. Paulista Tisiol.*, 20 (1959) 247.